

ROMER LABS
FLUOROQUANT AFLA (FQ AFLA)
FOR AFLATOXIN TESTING

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GENERAL INFORMATION

The FluoroQuant Afla test kit is a rapid, quantitative fluorometric test for detection of total aflatoxins (B₁, B₂, G₁, and G₂) using a solid phase extraction (SPE) column for sample purification. Samples are extracted with 80% methanol (MeOH)/20% deionized or distilled water (v/v), and then passed through the SPE column. The column retains impurities and allows purified aflatoxins to pass through. Eluent is placed into a clean tube. Developer solution is added to the eluent, which causes aflatoxin to fluoresce. The sample is then read in a calibrated FQ Reader.

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@usda.gov.

Approved Test Kit Information

Test Kit Vendor:	<i>Romer Labs, Inc. (636) 583-8600</i>
Test Kit Name:	FluoroQuant Afla
Product Number:	COKFA1010
Effective Date of Instructions:	05/11/2015
Instructions Revision Number	0
Conformance Range:	5 – 100 ppb
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	Yes
Approved Commodities:	Corn, brewers rice, brown rice, corn meal, corn/soy blend, milled rice, popcorn, rough rice, sorghum, soybeans, and wheat
Extraction method:	Blend 50-gram sample with 100 milliliters (mL) 80% MeOH/20% deionized or distilled water (v/v) for 1 minute. For corn/soy blend and soybeans blend 50-gram sample with 200 mL 86% Acetonitrile/14% deionized or distilled water (v/v) for 0.5 minute.
Test Format:	Solid phase extraction cleanup with fluorometric detection
Detection Method:	FQ Reader (Product Model No. EQFFM3010)

PREPARATION OF TESTING MATERIALS AND EXTRACTION SOLVENT

a. Preparation of Developer Solution:

- (1) Transfer the contents of one developer concentrate vial into 25 mL of distilled or deionized water using the disposable transfer pipette. If the developer concentrate is not yellow / brown when pulled into the disposable pipette, discard and use another vial.
- (2) Rinse the empty vial three times with the prepared developer, returning the rinse to the 50 mL amber bottle each time.
- (3) Developer must be made fresh a minimum of every 24 hours.
- (4) It is acceptable to make a “double batch” (2 vials to 50mL water) if a large number of samples are anticipated.

NOTE: All reagents and kit components must be at room temperature 20-24°C (68-75°F) before use.

b. Calibration of FQ Reader Fluorometer:

Calibration of FQ Reader Fluorometer should be completed at least once in every 24 hours.

- (1) Turn on the FQ Reader Fluorometer. The Test Kit Selection Screen will appear (no warm-up period is required). The test kit selection screen shows all test kits that are available for reading on the instrument.
- (2) Touch “FluoroQuant Afla”. The Commodity selection screen will appear automatically.
- (3) Ensure that the correct Optical Kit is installed in the FQ Reader for the test to be run. The FQ Module A should be installed for use with FluoroQuant Afla test kits.
- (4) Touch the commodity name that you are analyzing. For example, for corn, touch “Corn”. The Calibration screen will appear automatically following the selection of the commodity to be analyzed.

NOTE: Brewers rice is run on the “milled rice” calibration. Brown rice and rough rice are run on the “corn” calibration

- (5) Insert High calibrator when instructed; close the lid and touch “OK”.
- (6) Insert Low calibrator when instructed; close the lid and touch “OK”.
- (7) The Home screen appears following a successful calibration. The FQ Reader will display the message “Calibration Complete! Ready to Begin Testing”.
- (8) Insert Control (yellow) calibrator and close the lid when calibration is completed. Read the control calibrator and check the value against the range listed on the card. The value must be within the range listed on the card in the calibrator box to proceed with running samples.

c. **Preparation of Extraction Solvent A: 80% MeOH/20% deionized or distilled water (v/v) (for Brewers Rice, Brown Rice, Corn, Corn Meal, Milled Rice, Popcorn, Rough Rice, Sorghum, and Wheat):**

NOTE: 80/20 MeOH/water may be purchased pre-mixed. It can also be prepared by following the procedure below.

- (1) Using a 1000 mL graduated cylinder, measure 800 mL of methanol (ACS grade or better) and place it into a clean carboy with spigot.
- (2) Using a 250 mL graduated cylinder, measure 200 mL deionized or distilled water and add into the methanol and shake until it is completely mixed.
- (3) Label the container stating the mixture (80 percent methanol and 20 percent water), date of preparation, and initials of technician who prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container until needed. The solution should be stored for a maximum of 12 months.

d. **Preparation of Extraction Solvent B: 86% Acetonitrile/14% deionized or distilled water (v/v) (for Corn/Soy Blend and Soybeans):**

NOTE: 86/14 acetonitrile/water may be purchased pre-mixed. It can also be prepared by following the procedure below.

- (1) Using a 1000 mL graduated cylinder, measure 860 mL of acetonitrile (HPLC grade) and place it into a clean carboy with spigot.
- (2) Using a 250 mL graduated cylinder, measure 140 mL deionized or distilled water and add into the methanol and shake until it is completely mixed.
- (3) Label the container stating the mixture (86 percent acetonitrile and 14 percent water), date of preparation, and initials of technician who prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container until needed. The solution should be stored for a maximum of 12 months.

EXTRACTION PROCEDURES AND SAMPLE PREPERATION

a. **Extraction Procedure for Brewers Rice, Brown Rice, Corn, Corn Meal, Milled Rice, Popcorn, Rough Rice, Sorghum, Wheat:**

- (1) Weigh out 50 grams (± 0.2 g) of ground sample into a glass or polypropylene blender jar.
- (2) Add 100 mL of extraction solvent A and close the jar securely using a blender blade assembly.
- (3) Blend the sample on high for 1 minute.

- (4) Filter the sample using coffee filter into a glass or polypropylene container and label with the sample identification (do not use polyethylene container or any other type of plastic).
- (5) The filtered extract is now ready for assay (can be used for next 24 hours if sealed tightly).

b. Extraction Procedure for Corn/Soy Blend and Soybeans:

- (1) Weigh out 50 grams (± 0.2 grams) of ground sample into a glass or polypropylene blender jar.
- (2) Add 200 mL of extraction solvent B and close the jar securely using a blender blade assembly.
- (3) Blend the sample on high for 30 seconds.
- (4) Filter the sample using coffee filter into a glass or polypropylene container and label with the sample identification (do not use polyethylene container or any other type of plastic).
- (5) The filtered extract is now ready for assay (can be used for next 24 hours if sealed tightly)

TEST PROCEDURE

a. Analysis Procedure for Brewers Rice, Brown Rice, Corn, Corn Meal, Milled Rice, Popcorn, Rough Rice, Sorghum, and Wheat:

- (1) Pipet 1000 microliters (μL) of sample extract into the top of a SolSep 2001 cleanup (SPE) column.
- (2) Add 1000 microliters (μL) of the diluent (diluent is test kit lot specific) to the extract in the SolSep 2001 cleanup column and mix well by pipetting up and down three times. The solution may be difficult to push through the column if the liquids are not mixed well.
- (3) Place the SolSep 2001 cleanup column into a 12 x 75 mm cuvette.
- (4) Insert the syringe barrel and stopper into the top of the column.
- (5) Push the extract completely through the column until air comes out of the bottom of the column. (Alternate method: Use manual or powered vacuum column stand to push extract through column).
- (6) Transfer 500 microliters (μL) of the purified sample into a clean 12 x 75 mm cuvette (provided with the test kit).

b. Analysis Procedure for Corn/Soy Blend and Soybeans:

- (1) Pipet 2000 microliters (μL) of sample extract into the top of a SolSep 2001 cleanup (SPE) column.
- (2) Place the SolSep 2001 cleanup column into a 12 x 75 mm cuvette.
- (3) Insert the syringe barrel and stopper into the top of the column.
- (4) Push the extract completely through the column until air comes out of the bottom of the column. (Alternate method: Use manual or powered vacuum column stand to push extract through column).
- (5) Transfer 1000 microliters (μL) of the purified sample into a clean 12 x 75 mm cuvette (provided with the test kit).

c. Reading the Results

- (1) The Home screen of the FQ Reader will display the test kit in use (center gray box at top), the commodity for which the instrument has been calibrated (green box at top right), and the results of any measurements that have been performed (up to the previous 20 measurements).
- (2) Touch "Sample ID" to name the next sample to be analyzed (optional). Using the keypad, enter the sample name into the name field. Touch "Save" to save the sample ID.
- (3) Add 1000 μL of prepared developer to the purified extract.
- (4) Cap the cuvette and vortex for 5 seconds.
- (5) Wipe the cuvette with lint-free paper or cloth (included with FQ Reader) and insert into the calibrated fluorometer.
- (6) The FQ Reader will begin a 15 second countdown when the lid is closed. If the countdown does not begin, touch the large green read button in the upper right hand corner of the screen to begin. (The first reading after calibration or after the fluorometer has awakened from sleep mode will not begin the countdown automatically.)
- (7) After a preprogrammed 15 second delay, the results will be displayed and printed in part per billion.

Note: Once the developer is added to the purified sample, it must be mixed and analyzed immediately.

d. Interpretation of Results:

- (1) Results will be displayed on the FQ Reader in parts per billion total aflatoxins.

SUPPLEMENTAL ANALYSIS:

Supplemental analysis is not required up to 1000 ppb quantitation of aflatoxins. However, any sample containing greater than 1000 ppb aflatoxins can be quantified by diluting the sample extract as described below.

- (1) Dilute the sample extract 1 to 10 with extraction solvent A (e.g., add 1mL filtered extract into 9 mL extraction solvent A. Dilution factor is 10.
- (2) Follow the analysis procedure for corn under the “**TEST PROCEDURE**” on page 4.
- (3) Multiply the final result by 10. This extends the range to 10,000 ppb.

REPORTING AND CERTIFYING TEST RESULTS

Refer to the current instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@udsa.gov).

STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions

- (1) Store test kits at 18-25°C (64-77°F) when not in use, and do not use beyond the expiration date. Do not freeze and do not leave in direct sunlight.
- (2) Cuvettes should remain sealed in original box until ready for use. The sealing film on top of the cuvettes should remain in place during storage to prevent dust from entering.

b. Precautions

- (1) All reagents must be at room temperature before the assay is run.
- (2) Adhere to the GIPSA-Issued instructions of the test procedures.
- (3) Do not re-use columns.
- (4) Consider all materials, containers and devices that are exposed to the sample to be contaminated with toxin. Wear protective gloves and safety glasses when using the kit.
- (5) The components in this test kit have been quality control tested as a standard batch unit. Do not mix components from different lot numbers.

EQUIPMENT AND SUPPLIES

a. Materials Supplied with the Kit:

- (1) 1 bag containing 25 SolSep 2001 cleanup columns
- (2) 1 box containing 50, 12 x 75 mm cuvettes; 1 bag of cuvette caps; 1 bag of pipette tips
- (3) 1 bag of coffee filter paper; 1 bag of 8 disposable transfer pipets
- (4) 1 bottle of diluent (HPLC Grade Acetonitrile); 1 box of 8 vials of developer

b. Materials Required but not Provided with Kit

- (1) 80/20 Methanol/Water extraction solvent
- (2) 86/14 Acetonitrile/Water extraction solvent (for corn/soy blend and soybeans)
- (3) EQMMS2010: Romer Series II Mill or equivalent; EQOLE1010: Balance, 400 grams
- (4) EQOLE1020: Blender; EQOLE1025: Blender jars – ½ pint (8 oz.)
- (5) EQOLE1050: 100 mL Graduated cylinder and COOLS1149: 250 mL Graduated cylinder
- (6) EQOLE1130: Extraction jars or containers; EQOLE1301: Timer; EQOLE1350: Funnel

c. Assay Procedure

- (1) Distilled or deionized water
- (2) EQOLE1050: Fixed 1000 µL pipette; EQOLE1160: Fixed 500 µL pipette
- (3) EQOLE1210: Test tube rack; EQOLE1241: Repipettor
- (4) EQOLE1335: Vortex mixer; EQFFM3010: FQ Reader with printer
- (5) COKFA2040: High, Low, and Control fluorometer calibrator ampoules
- (6) COKFA1081: Syringe plunger and stopper assembly OR COKFA1085: Optional plunger stand

REVISION HISTORY

Revision 0 (05/11/2015)